MODULAR GRAFTED POLYMERIC SURFACES

CROSS-REFERENCE(S) TO RELATED APPLICATION(S)

This application claims the benefit of U.S. Provisional Patent Application No. 60/218,236 filed July 14, 2000 and U.S. Provisional Patent Application 5 No. 60/282,099 filed April 6, 2001 (both of which applications are incorporated herein by reference in their entireties).

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to new surfaces for solid phase 10 chemistry applications, more specifically plastics surfaces modified by graft polymerization for use in chemical synthesis and/or immobilization of chemical entities and/or compounds.

Description of the Related Art

Over the past decade, the simultaneous synthesis of large numbers of 15 compounds, either as discrete members of sets, or as mixtures, has become an important tool in the drug discovery process in the pharmaceutical industry. Whether achieved by manual or automated means, via solution phase or solid-supported (solid phase) synthetic methodologies, this new area of chemical sciences is generally referred to as "combinatorial chemistry". Sets of compounds prepared by these methods are generically 20 known as "combinatorial libraries".

The rise in the popularity of combinatorial chemistry has paralleled the development of high throughput screening technologies, which now allow hundreds to thousands of assays to be run concurrently, hence taxing the abilities of traditional medicinal chemistry programs to supply new compounds for the drug discovery process. 25 High throughput screening has become viable due to dramatic developments in robotics

and computation, coupled with the equally dramatic developments in molecular biology. Over the past few years, large numbers of receptors have been identified and characterized. Furthermore, many receptors can now be expressed on the surface of cells; these cells can be plated out into convenient array formats, hence facilitating automated screening for the

5   ability of test compounds to bind to the receptor and/or modulate the activity mediated by the receptor.

The original "solid phase" used in these methods was beaded polystyrene cross-linked with divinylbenzene. Over the years, polymethylacrylamide (*e.g.*, PEPSYNK^{TM}), polystyrene-polyethylene glycol copolymers (*e.g.*, RAPP TENTAGEL^{TM}),

10   macroporous polystyrene (*e.g.*, Polymer Laboratories Stratospheres) and many other "solid phases" have been developed. Many of these "resins" were designed to service the peptide and oligonucleotide synthesis market. However, there are limitations in using such resins, including the need for a containment strategy for synthesis of compounds in quantity, the different reaction profiles between different bead sizes due to increased diffusion

15   pathlength and increased cross-linking with larger resin beads, differential swelling in different solvents, with some resins having 8-fold differences in volume and limited temperature stability, with a maximum of 150°C for the best performing resins.

Another type of solid phase that has become available more recently is a pellicular type of solid support where a more mobile polymer is grafted to rigid plastics

20   (hereinafter referred to as "grafted supports"). Compared to the resins that dominate this field, grafted supports allow great flexibility of design as plastics are available as sheets, films or threads, or can be molded into any shape as required. Many different polymers or co-polymers can be grafted on to any particular shape to give a wide choice of options in the physicochemical characteristics of the actual solid support. Unlike resins, it is the

25   surface area of the grafted support and not the volume that determines loading capacity. It is possible to achieve consistency of reaction kinetics between grafted supports of different sizes and shapes. This is difficult with resins, because diffusion pathlength must change with size and, as a consequence, reaction rates must change. Other advantages include an integrated design of the solid phase used in synthesis with a method of containment into

"resin packets" and minimal swelling in different solvents as the majority of the substrate polymer does not swell.

The limitations with the current grafted supports include the temperature limit of about 120°C, the loading per unit volume is lower than the best high loading resins and the perceived reaction rate is slower than conventional resins.

Traditionally, pharmaceutical companies have screened their large compound inventories, which have been built up over many years, to discover new lead compounds. These inventories have been built up from a number of sources, including natural products and synthetic intermediates. However, it has been the labor of large numbers of medicinal chemists, working over many decades, which has been largely responsible for the compound collections. Medicinal chemists have generally prepared small sets of compounds within a target family and obtained preliminary screening data using a limited range of screening assays before proceeding to make further compounds in that family.

With its ability to provide more compounds per unit time and per unit chemist, and with the increasing difficulty of finding new lead compounds, combinatorial chemistry is becoming increasingly widely utilized, using either solution phase or solid phase techniques. Hence the number of compounds produced by a typical chemist in a year may be increased by orders of magnitude.

Combinatorial chemistry represents an important paradigm shift in the practice of synthetic chemistry. Over the past few years, enormous advances have been achieved in the ability of chemists to enhance the synthesis and design of molecules that fit a specific set of parameters, rather than just indiscriminately and wastefully synthesizing a vast number of compounds. Currently, there are two schools of thought as to the scale of synthesis required for library synthesis. While there is no doubt that the trend in compound library generation is towards well-designed, individual, pure and fully characterized compounds, there is still a split in the scale of synthesis between large scale (20mg/compound) and small scale (0.5-1mg/compound) syntheses.

3

Despite the success and advantages of solid phase organic chemistry, there are still limitations to this technique. Firstly, the reactions can be slow relative to their solution phase counterparts, although the development of high-performance polyethylene glycol-based resins and grafted polymeric supports has alleviated this problem to a certain

5   extent.

Secondly, it can also be difficult to monitor reaction progress. Considerable improvements have been made in this area in recent years, with the use of techniques such as Fourier transform infra-red spectrometry, matrix-assisted laser desorption inteferometry-mass spectrometry, Gelphase and Magic Angle Spinning (MAS) nuclear magnetic

10 resonance spectrometry etc. However, these techniques still do not provide the same quality of analysis as rapidly and conveniently as conventional solution-phase techniques such as thin layer chromatography, gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, SFC-mass spectrometry, nuclear magnetic resonance spectrometry, etc. This problem has been partly addressed recently with the use of grafted

15 polymer surfaces in MAS-nuclear magnetic resonance monitoring of solid phase reactions (Sefler and Gerritz, J. Com. Chem. 2000, 2, 127-133).

Thirdly, one of the most time-consuming aspects of solid-phase organic chemistry is usually the process of attempting to optimize solution-phase chemistry on a polymer-supported substrate, particularly where a long synthetic sequence is required.

20 Over the last few years increased emphasis has been placed on the development of parallel solution phase methods of combinatorial library synthesis. Solution-phase synthesis has obvious advantages: experience gained over 100 years of chemical reaction experience is available, reactions can be monitored using standard techniques, and no additional steps are required to attach and detach compounds from the

25 solid support.

In the past, solution-phase parallel synthesis was usually only applied to short, high-yielding reaction sequences, because of the difficulties in purifying large numbers of reaction products simultaneously. These problems have been recently addressed, with the development of specifically-fictionalized resins designed to react with

and remove unreacted reagents from a reaction mixture. These so-called polymer-supported reagents and scavenger resins have revolutionized solution-phase parallel synthesis.

Supported reagents are reactive species that are associated with a support material. They transform a substrate(s) to a new chemical product(s), and the excess or spent reagent may be removed by filtration.

Supported catalysts are reactive species that are associated with a support material. They are used in sub-stoichiometric quantities to transform a substrate(s) to a new chemical product(s), and may be removed by filtration and recycled

Supported scavengers are reactive species that are associated with a support material. They selectively quench or sequester by-products of the reaction, or remove excess or unreacted starting materials, and may be removed by filtration.

In this way the advantages of solid-phase synthesis, *i.e.*, the ability to use excess reagents to drive reactions to completion, and the ease of product isolation, have become applicable to solution-phase synthesis. A crucial advantage for industrial processes is that toxic, noxious or hazardous reagents and their by-products can be immobilized, and are therefore not released into solution, thereby improving their general acceptability, utility and safety profile. In addition, for reactions that are low-yielding and result in complex mixtures of products, scavengers can be used to isolate pure products in a simple fashion, without the need for tedious conventional work-up and purification procedures. Simple work-up procedures such as filtration and solvent removal make solid-supported reagents and scavengers particularly attractive for combinatorial library generation.

As the number of compounds to be synthesized increases, so too does the complexity of handling the resins in parallel. Thus a fundamental limitation to this method is that the solid phase needs to be removed in a high-throughput parallel fashion, thus negating some of the advantages of using resins. Expensive equipment such as robotic automation is generally required to remove the resin. In addition, because resins swell in solvent, large excesses of solvents are required to eliminate the possibility of the reaction

solutions becoming essentially dry. Organic solvents may be toxic, and their disposal presents a significant environmental problem.

Affinity separation of molecules, whether large or small, requires a fundamental understanding of how the molecule(s) of interest interact with the solid-phase

5   matrix. These fundamental interactions are all chemically based, and involve the interaction of chemical functional groups. An understanding of how different functional groups interact between the solid and solution phase enables the prediction of how specific proteins will react with specific solid-phase surfaces.

In particular, it has been found that certain specific plastics and modified

10   forms thereof can be used as a solid support for multiple parallel organic compound synthesis and several other related applications. In addition, it has also been found that significantly improved efficiency of synthesis can be achieved by using reagents and scavengers bound to a solid-phase support that is a modular grafted polymeric surface. The use of a modular grafted surface overcomes the disadvantages listed above.

15   Accordingly, there is a continuing need in the art for new and/or improved surfaces for use in solid phase organic synthesis, as well as for capture, presentation or preparation of biomolecules. The present invention fulfills these needs, and provides further related advantages.

SUMMARY OF THE INVENTION

20   In brief, this invention is generally directed to an activated modular grafted polymeric surface, which is suitable for use as a reagent for solid phase organic synthesis, or as a reagent for the affinity capture, presentation or preparation of biomolecules such as proteins, oligonucleotides, nucleic acids, peptides, and lectins. The grafted polymeric surfaces of the invention are particularly useful as scavenger reagents in combinatorial

25   synthetic protocols, and as affinity reagents in protein purification and proteomics.

In a first aspect, the invention provides an activated modular grafted polymeric surface. The term "grafted polymeric surface" refers to a polymer which has

6

been modified by graft polymerization. Derivatives, blends and copolymers thereof modified by graft polymerization are also within the scope of the invention.

The grafted polymeric surface of the invention is useful as a reagent for solid phase organic synthesis, or as a reagent for the affinity capture, presentation or preparation of biomolecules such as proteins, oligonucleotides, nucleic acids, peptides, and lectins. The term "solid phase chemistry" is used herein in its broadest sense, and refers to the use of solid supports that are insoluble materials to which chemical entities and/or compounds attach during various chemical applications.

In one embodiment, the polymer is a co-polymer of polyethylene and polypropylene.

In a second embodiment, the polymer is a branched polyolefin, or a derivative, blend or copolymer thereof modified by graft polymerization. This is referred to herein as a "modified branched polyolefin"; this is particularly preferred when the graft is polyacrylic acid. This modified branched polyolefin is used *per se* and represents a second aspect of this invention.

Preferably the branched polyolefin is a polyalkylalkene, more preferably poly-(4-methylpentene-1), referred to herein as "PMP". The trade name for PMP manufactured by Mitsui Chemicals Inc(3-2-5 Kasumigaseki Chiyoda-ku, Tokyo 100, Japan) is TPX.

Suitable types of graft polymerization include gamma-irradiation graft polymerization, ozone-induced graft polymerization, plasma-induced graft polymerization, UV-initiated graft polymerization and chemical-initiated graft polymerization, such as peroxide-initiated graft polymerization.

The polymers which may be grafted on to the branched polyolefin include polyvinyls, polyvinylalcohols such as polyvinyl acetate, polyacrylates such as polyacrylic acids, polymethacrylates such as 2-hydroxyethyl methacrylate (HEMA), polyacrylamides such as dimethylacrylamide (DMA), polyethylkene glycols, polylactic acids, and derivatives, blends and copolymers thereof. In one preferred embodiment the graft polymer is polystyrene. In a further embodiment, the graft polymer is a polyvinylalcohol.

7

The term "activated" refers to a grafted polymeric surface to which is bound a reagent such as triphenylphosphine, a reductant or oxidant, a chelating metal such as nickel or calcium, a scavenger such as a nucleophilic group, e.g., aminomethyl or hydrazino, or an electrophilic group, e.g., isocyanate, tosyl chloride, or benzaldehyde, or a

5 catalyst such as dimethylaminopyridine. A chelating metal is preferred when the graft is polyacrylic acid.

The term "modular" means that the activated grafted polymeric surface is in the form of a plurality of physical units which are suitable for use in a set of simultaneous chemical reactions, and which provide reproducible chemical properties. These may be of

10 a wide variety of desired shapes, such as lanterns, gears, pins, pucks, discs, beads, microtitre plates, sheets, etc. It will be appreciated that the activated grafted polymer may be molded into any shape, depending on the desired application. This also provides flexibility in the physiochemical properties of the activated grafted polymeric support, and means that a specialized containment apparatus is not required, in contrast to the use of

15 resins.

The activating moiety may be aldehyde, carboxylate, amino, hydroxide, biotin, thiol, tosyl acid, tosyl chloride, hydrazino, isocyanate, or any other chemical moiety which could be used with appropriate chemistry to act as a chemical scavenger, solid-supported reagent, solid-supported catalyst, or affinity capture agent for proteins.

20 Preferably the activating moiety is aldehyde.

Preferably the grafted polymeric surface is able to bind a target agent which is an amine compound capable of Schiff base formation. In one embodiment, the target agent is a biotinylated molecule, such as a peptide, protein, oligonucleotide, lipid or sugar. Alternatively the target agent is a protein, such as streptavidin, or an enzyme, for example

25 horseradish peroxidase.

Optionally there may be one or more spacer sequences, which may be the same or different, between the aldehyde and the derivatized polymer support.

In further embodiments, the invention provides:

8

(a)   a benzaldehyde polystyrene lantern, which can be used for example to scavenge phenylhydrazine;

(b)   a benzaldehyde polystyrene lantern, coupled to streptavidin or horseradish peroxidase; and

(c)   a nickel-chelating polyacrylic acid gear, which can be used for affinity separation of hexahistidine-tagged proteins.

It will be appreciated that the protein affinity capture agents of the invention are particularly suitable for use in in proteomic applications.

These and other aspects of the invention will be evident upon reference to the following detailed description and attached figures. To this end, all documents cited herein are hereby incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 is a diagram showing the dimensions of the PMP Gears.

Figure 2 is a photographic representation of nickel-chelated polyacrylic acid gears according to the invention (PMP gears), showing from left to right:

(1)   Gear grafted with acrylic acid (2);

(2)   Gear as in (1) stained with Rhodamine B dye;

(3)   Gear as in (1) doped with nickel chelating metal; and

(4)   Gear as in (3) stained with 1% rubeanic acid.

DETAILED DESCRIPTION OF THE INVENTION

The invention will now be described in detail by way of reference only to the following non-limiting examples and drawings.

In addition to the ability of the grafted polymeric surfaces of the invention to provide flexibility of modular shapes, other advantages of the polymeric surfaces of the invention, particularly the branched polyolefin, include:

- high heat-resistance, with a melting point of about 220°C to about 240°C, which means that it can withstand reaction temperatures up to about 200°C;

this covers the temperature range used in most organic chemistry applications;

- excellent transparency, with its transmittance of visual light being >90% and of ultraviolet light being about 300 nm to about 400 nm; this makes it useful in applications relying on color;
- excellent chemical resistance;
- good heat aging and resistance to steam or boiling water;
- high loadings of up to about 300 nmol per sq cm, resulting in superior yield properties compared to resins, where the maximum loading possible is about 124 nmol per sq cm;
- suited to both batch and flow type synthesizers; and
- when molded into microtitre plates, has the potential for use in small scale (<500 nmol) synthesis of small molecules (MW <500), peptides and oligonucleotides.

There are two advantages to solid-phase chemistry. Firstly, isolation of support-bound reaction products is accomplished simply by washing away reagents from the support-bound material, and therefore reactions can be driven to completion by the use of excess reagents. Secondly, innovative methods are available for the manipulation of discrete compounds and for tracking the identity of compounds when compounds are attached to a solid support.

Examples of solid phase chemistry applications in which the polymers of the invention are particularly useful include chemical synthesis, scavenging, purification, immobilization and/or chelation.

The use of grafted surfaces for preparation of solid phase reagents and scavengers presents significant advantages, since

(a)   Grafted surfaces can be molded into many different and desirable shapes, such as lanterns, gears, pins, pucks, beads, discs, microtitre plates, sheets, etc, which provide reproducible chemical properties;

(b)   The modular support does not swell, and therefore the volume of reaction solution can be confidently set prior to chemistry being performed;

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(c) Modular supports such as lanterns, gears, pins, discs, or pucks can be mounted in an 8 x 12 or similar format and simply lowered into a reaction solution, allowed to react, and then lifted clear of the solution, in a simple but parallel fashion.

The chemical synthesis applications include synthesis of organic compounds, in particular biological organic compounds such as peptides, proteins and oligonucleotides.

The higher loading capacity of the polymers of the invention, particularly the branched polyolefin and the modified branched polyolefin of the invention, means that they are suitable for use in scavenger applications, *i.e.*, the removal of unwanted by-products from chemical reactions (Thompson, L.A., *Recent Opinions in Chemical Biology*, 2000, *4*, 324-337). Specialist filtration equipment is also not required in order to perform the scavenger reactions using the grafted modular polymeric supports of the present invention.

Another important application is as a solid phase fluorescence quenching assay for use in determination of substrate as well as inhibitor specificity of proteolytic enzymes (St Hilaire, P.M., Willert, M., Juliano, M.A., Juliano, L., Meldal, M., *J. Comb. Chem.*, 1999, *1*, 509-523).

The high loading coupled with the high kinetics obtained from the grafted modular polymeric supports of the present invention makes the polymer an excellent support for affinity chromatography applications. Purification of proteins from proteomic mixtures and purification of DNA from genomic mixtures are two broad areas of application. For example, grafting of polyacrylic acid to branched polyolefin gives a surface which can chelate nickel ions. This surface can then be used to affinity capture proteins which have been genetically engineered to possess a multi-histidine tag such as hexa-histidine. The histidine tag coordinates with the nickel surface.

Branched polyolefin grafted with acrylic acid results in a surface bearing carboxylic acid groups. This surface binds not only metals such as nickel, as described above, but also other metals, for example, copper, zinc, cadmium, silver, palladium, platinum, gold and lead. Thus the modified branched polyolefin has application in removal

11

of metals from the environment, for example in the cleaning of contaminated sites, or in the prevention of pollution, for example in the treatment of industrial waste water before release into effluent streams or in the treatment of sewage.
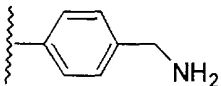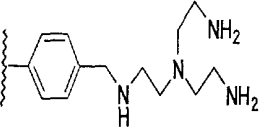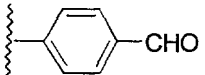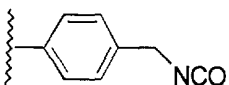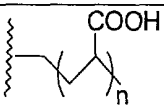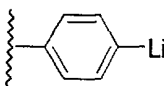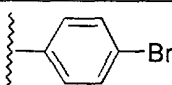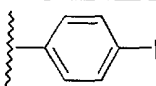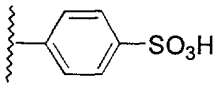
Although this invention is specifically described with reference to the use of
5   particular modular shapes and combinations of polymer and activating groups, it will be clearly understood that lanterns, gears or other modular shapes activated by other methods, such as carboxylate, amino, hydroxide, biotin, or any other chemical moieties which could be used with appropriate chemistry either as solid phase reagents or scavengers, or as affinity surfaces for capture, presentation or preparation of biomolecules such as proteins,
10   oligonucleotides, nucleic acids, peptides, and lectins, are suitable for use in the invention.
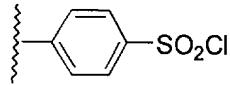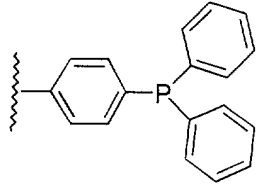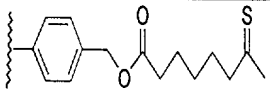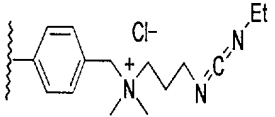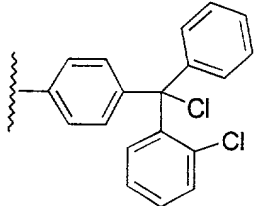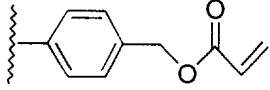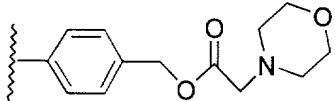
For many years, a multipin array system has been used for solid-phase combinatorial peptide synthesis. This system is marketed by Mimotopes Pty Ltd, Clayton, Australia, and is used for synthesizing peptides and for synthesizing peptide libraries. The proprietary pin, Crown^{TM} and SynPhase^{TM} Lantern support systems utilize polyethylene or
15   polypropylene copolymers grafted with 2-hydroxyethyl methacrylate polymer (HEMA), methacrylic acid/dimethylacrylamide polymer(MA/DMA) or polystyrene (PS) (*see, e.g.,* Maeji et al., *Reactive Polymers* 1994, *22*, 203-212).

Examples of reagents that may be used in the invention and the purposes for which they are particularly suitable are shown in Table 1. It will be clearly understood that
20   this list is representative only, and is not intended to be exhaustive.

Table 1

Grafted Polymer-bound Reagents and Scavengers

Structure	Compound Designation	
(structure with NH$_2$)	1	Scavenger nucleophilic
(structure with NH$_2$, N, N, NH$_2$, N H)	2	Scavenger nucleophilic
(structure with CHO)	3	Scavenger electrophilic
(structure with NCO)	4	Scavenger electrophilic
(structure with COOH, n)	5	Metal chelation surface
(structure with Li)	6	Reagent Precursor to numbers 7, 8, 11 and 14
(structure with Br)	7	Reagent Precursor to 11 and 14
(structure with I)	8	Reagent Precursor to 11 and 13
(structure with SO$_3$H)		Reagent Ion-exchange

13

Structure		
⌇—⟨C₆H₄⟩—SO₂Cl	10	Scavenger electrophilic
⌇—⟨C₆H₄⟩—P(C₆H₅)₂	11	Reagent
⌇—⟨C₆H₄⟩—CH₂—O—C(O)—(CH₂)₄—C(S)—CH₃	12	Reagent
⌇—⟨C₆H₄⟩—CH₂—N⁺(CH₃)₂ Cl⁻ ...—C≡N—N(Et)	13	Reagent
⌇—⟨C₆H₄⟩—C(C₆H₅)(Cl)(o-Cl-C₆H₄)	14	Linker
⌇—⟨C₆H₄⟩—CH₂—O—C(O)—CH=CH₂	15	Scavenger electrophilic
⌇—⟨C₆H₄⟩—CH₂—O—C(O)—CH₂—N(morpholino)	16	Reagent

EXAMPLE 1

GRAFTING OF 1000 PMP "GEARS"*

Styrene (48 mL) was mixed with methanol (112 mL). The mixture was then
5   poured into a bottle containing 1000 Gears. The mixture was purged with nitrogen for 20
minutes. The bottle was capped tightly and then placed in the irradiator machine. The
contents were irradiated at a dose rate of 1.6 kGy per hour for 7 hours with occasional
shaking of the bottle. The bottle was removed, the styrene drained and the contents washed
with dichloromethane (5 x 10 minutes). The Gears were removed and air dried before
10   being derivatized.

*"Gears" refer to a specific molded shape of PMP (see Figure 1 for
preferred dimensions).

14

EXAMPLE 2

## SOLID PHASE SYNTHESIS OF 4-CHLORO-3-[3-(4-TRIFLUOROMETHYL-PHENYL)-UREIDO]-BENZAMIDE

5   The grafted polystyrene gears of Example 1 were aminomethylated and derivatized with a Rink linker, using standard methods as described by Adams *et al* (Adams, J.H., Cook, R.M., Hudsen, D., Jammalamadaka, V., Lyttle, M.H., and Songster, M.F., *J. Org. Chem.*, 1998, *63*, 3706-3716), and shown in Scheme 1 below.

Scheme 1

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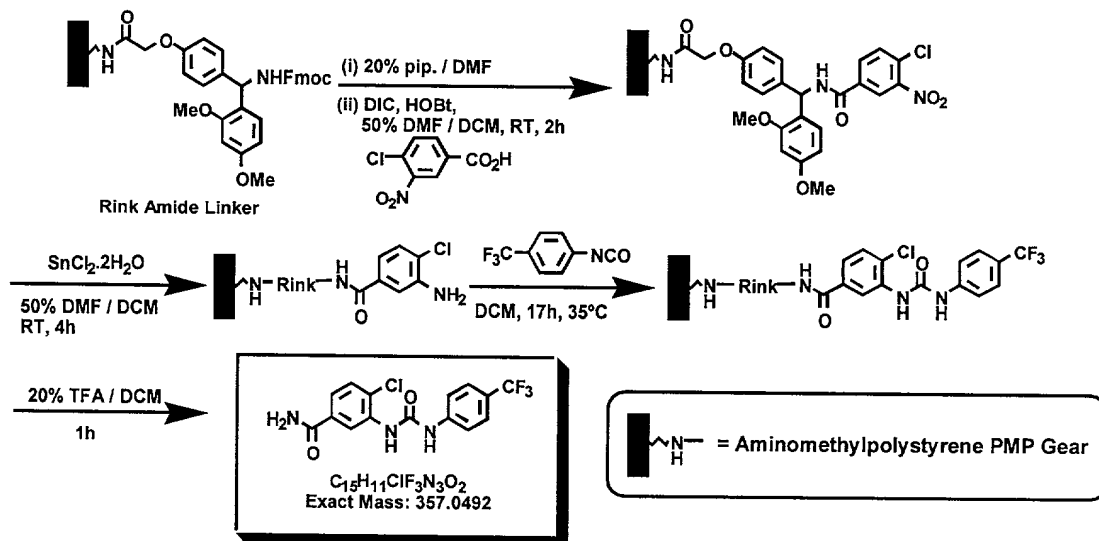
Gears (loading = 9μmol) were covered with 20% v/v piperidine in DMF and
25   stood at room temperature for 30mins. The solution was decanted and the gears washed in turn with DMF (2 x 3 mins) and DCM (2 x 3 mins). Each Fmoc-deprotected gear was treated with 0.5mL of a solution of DIC (0.2M, 100μmol, 2.9 mole equivalents), HOBt (0.2M, 100μmol, 2.9 mole equivalents) and 4-chloro-3-nitrobenzoic acid (0.2M, 100μmol, 2.9 mole equivalents) in 50% v/v DCM/DMF at room temperature for 2h. The solution
30   was decanted and the gears washed in turn with DMF (2 x 3 mins) and DCM (2 x 3 mins).

Each gear was treated with 0.5mL of a 2M solution of tin (II) chloride dihydrate (1000µmol, 29 mole equivalents) at room temperature for 4h. The solution was decanted and the gears washed in turn with DMF (2 x 3 mins) and DCM (2 x 3 mins). Gears were pre-dried in a vacuum oven at 40°C/approximately 1mmHg for 18h. Each gear was treated

5   with 0.5mL of a 0.5M solution of 4-trifluoromethylphenylisocyanate (250µmol, 7.4 mole equivalents) in anhydrous DCM at 35°C for 18h. The reaction was allowed to cool to room temperature and the solution decanted. The gears were washed in turn with DCM (1 x 3 min), DMF (2 x 3 min) and DCM (2 x 3 min) and air dried. Individual gears were placed in Labsystems™ 1.1mL polypropylene tubes and treated with 20% v/v TFA in DCM (0.6-

10 0.8mL) for 1h. The excess TFA and solvent were removed using a centrifugal evaporator. Further drying was achieved by placing in a vacuum oven at 40°C/approximately 1mmHg for 18h. A 'quantitative' yield of 4-Chloro-3-[3-(4-trifluoromethyl-phenyl)-ureido]-benzamide was obtained, based on the weight of crude product and the specified initial loading of the gear. Samples were dissolved in 90% v/v CH_3CN/H_2O for HPLC or

15 Electrospray mass spectrometry (ESMS) analysis and DMSO-d6 for ^1H NMR spectroscopy. Purity (>95% at 214nm) based on reverse phase HPLC (C18 column). Analytical HPLC was performed on a Waters chromatography system using a Ranin microsorb-mv (#86-200-F3) RP-18 column (100A, 3µm). The following conditions were used: buffer A=water (0.1% H_3PO_4); buffer B=90% acetonitrile/10% water (0.1% H_3PO_4);

20 linear gradient A to B from 1 to 11 min; flow rate = 1.5mL min^{-1}. Absorbances were recorded at 214 and 254 nm. HPLC purities were determined by peak area at 214 nm. ESMS: found 358.00 [M+H]; expected 358.00. ESMS analyses were performed on a Perkin Elmer Sciex API III mass spectrometer.

25   EXAMPLE 3

SYNTHESIS OF POLYSTYRENE-BENZALDEHYDE LANTERNS 3

Fresh dichloromethane (850 mL) was added to polystyrene lanterns (4000) in a 2L multi-necked round-bottomed flask, followed by dimethyl methyl ether (27.3 mL, 300mM). Dimethyl methyl ether is highly toxic, and should only be used in a fumehood.

Standard precautions must be taken in washing and disposing of equipment and reagents. The contents of the flask were mixed with mechanical shaking under a nitrogen atmosphere. SnCl$_4$ (15.6 mL, 150 mM) was slowly added using a dry glass syringe. SnCl$_4$ is highly air sensitive, and should be bubbled with nitrogen before use. The mixture was reacted for 5 hours at room temperature, and drained from the lanterns into a beaker containing methanol (1 L). The lanterns were washed with dichloromethane (2 x 10 mins), followed by hot 20% H$_2$O/THF (1 hour), and soaked in 20%H$_2$O/THF overnight, then soaked in dichloromethane (30 min), and air dried. The product is polystyrene benzaldehyde lanterns 3.

EXAMPLE 4

USE OF POLYSTYRENE BENZALDEHYDE LANTERNS TO SCAVENGE PHENYLHYDRAZINE

Polystyrene benzaldehyde lanterns made as described in Example 3 (10 lanterns, 3 equivalents)) were added to a mixture containing phenylhydrazine (1 equivalent) in 1% acetic acid/ dichloromethane. The mixture was gently agitated for 1 hour at room temperature, after which thin-layer chromatography (solvent system: ethyl acetate, petrol, methanol: 10, 30, 5) indicated complete removal of the phenylhydrazine from the reaction mixture had occurred, yielding 2.

EXAMPLE 5

COUPLING PROTEINS TO BENZALDEHYDE POLYSTYRENE LANTERNS

The following is a further application of lanterns. A generic procedure for the coupling of proteins to lanterns is presented. As an illustration of the application two proteins were used, streptavidin (SA), and horseradish peroxidase (HRP). The proteins were coupled to the lantern by reaction of the aldehyde with each protein's amine groups to form Schiff's bases. Subsequent reductive amination forms a stable covalent link, with minimal leakage of the protein from the lantern.

In this example various derivatized lantern formats may be used; for example, there may be different spacer sequences between the aldehyde and the derivatized

17

polymer support, and in this example the term lantern will be understood to mean a lantern chemically derivatized to have an aldehyde moiety on the surface. A typical loading for a lantern is ≥15 μmole/lantern.

To demonstrate that protein could be bound to lanterns, the binding of the chosen proteins was tested on untreated lanterns as well as on lanterns treated to chemically block available aldehyde. Because of the nature of protein binding, it is also possible that protein may also bind to nominally blocked surfaces by non-specific absorption. To check for this non-specific binding, blocked lanterns were also prepared and tested.

Blocking procedure

Several lanterns were incubated for 1 hr at room temperature in a 1M Tris/HCl buffer pH 7.4 containing 0.2%w/v sodium cyano borohydride (Na B H_3CN).

The treated lanterns were then washed 5 times with water, and once with 0.01 M phosphate buffered saline, pH 7.4 (PBS).

Protein coupling

Two protein solutions were prepared, streptavidin (0.05 mg/mL containing with 0.2%w/v $NaBH_3CN$), and horseradish peroxidase (0.05 mg/mL containing 0.2%w/v $NaBH_3CN$). Lanterns and blocked lanterns were added to these protein solutions, and incubated for 2 hr at room temperature and then overnight at 4°C. All treated lanterns were then washed 5 times with water and once with PBS.

EXAMPLE 6

TESTING OF PROTEIN-TREATED LANTERNS

The streptavidin-treated lanterns were tested using an Enzyme Linked ImmunoSorbent Assay (ELISA). A monoclonal antibody for which biotinylated positive and negative binding peptides are available was used. Treated lanterns were divided into two groups, one of which was incubated in a PBS solution containing the "positive peptide" and the other in a PBS solution containing the "negative peptide". The ELISA

18

was according to the biotinylated peptide ELISA protocol described by Tribbick (Immunological Methods Manual 1996, Ch. 10.8, 816-826).

The average absorbance results are shown in Table 2.

Table 2
Results of ELISA Assay for Streptavidin

Lantern-SA-peptide	Absorbance
Positive peptide	1.2
Negative peptide	0.2

The horseradish peroxidase-treated lanterns were washed five times with water, and then individually placed into an aliquot of ELISA substrate. The protocol used was as described above. The average absorbance results are shown in Table 3.

Table 3
Results of ELISA Assay for Horseradish Peroxidase

Lantern	Absorbance
Lantern-HRP	1.35
Blocked Lantern-HRP	0.78

The results obtained for the two proteins illustrate the use of derivatized lanterns for the immobilization of proteins. The results obtained for HRP clearly show that enzyme activity was maintained, and that the protein was not denatured in the binding procedure.

The retention of functional streptavidin activity clearly indicates that the derivatized lanterns can be used to immobilize any biotinylated molecule, such as peptides, proteins, oligonucleotides, lipids and sugars. The use of the lanterns to immobilize streptavidin, avidin or any modified avidin, and in turn to immobilize biotinylated molecules, can be exploited in many applications by those skilled in the art.

EXAMPLE 7

SYNTHESIS OF NICKEL-CHELATED POLYACRYLIC ACID GEARS

Gears composed of polyacrylic acid-grafted poly-(4-methylpentene-1) were washed with water, 0.1M NaOH, water and then dosed with nickel solution (150mM

5   $NiSO_4.6H_2O$) for 30 minutes. The gears were then washed with water three times. This results in a pale green-colored gear. To confirm chelation of nickel to the polyacrylic acid graft, nickel-chelated gears were washed with 0.1M aqueous ammonium hydroxide and then reacted with 1% rubeanic acid in ethanol solution for 60 minutes. After washing with methanol, the gears remain black in color. This is illustrated in Figure 2.

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EXAMPLE 8

IMMOBILIZATION OF HEXA-HISTIDINE TAGGED RECOMBINANT PROTEIN ON NICKEL-

CHELATING POLYACRYLIC ACID GEARS CONTAINING DIFFERENT BASE POLYMERS

Three different injected molded base polymers were assessed for their

15   ability to graft acrylic acid and for their ability to immobilize a hexa-His tagged recombinant protein, SP22 (sperm protein 22) (Welch, J.E., Barbee, R.R., Roberts, N.L., Suarez, J.D., Klinefelter, G.R., *J. Androl.*, 1998, *19*, 385-393).

The three base polymers were:

(A)   poly-(4-methylpentene-1), otherwise known as "PMP";

20   (B)   a copolymer of polypropylene and polyethylene, otherwise known as "PMA"; and

(C)   high-density polyethylene, otherwise known as "HDPE".

All were grafted with acrylic acid acid and doped with nickel as described in Example 5 to obtain 6% polyacrylic acid gears.

25   In a 96 well plate, 200µL of 8M urea were added to each well, followed by 20µL of hexa-histidine labeled SP22 dissolved in 8M urea. The solutions were diluted by 11-fold into two consecutive wells. The three different types of acrylic acid-grafted gears described above were incubated in a 0.1M solution of $NiSO_4.6H_2O$ for one hour before being washed with water (x2). The nickel-coated acrylic acid gears were placed in the

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wells containing the diluted solutions of hexa-histidine labeled SP22. Reaction was allowed to proceed for 1 hour, after which the gears were removed and washed with water (x2). The gears were then placed in wells containing 200µL of a solution of antibody to hexa-Histidine SP22 (ELISA substrate). The ELISA protocol was according to the protocol described by Tribbick (op. cit). The average absorbance results are shown in Table 4.

Table 4

Results of ELISA Assay for hexa-histidine SP22

Type 6% Acrylic Acid	Volume of Recombinant Protein Solution (µL)	Ni^{2+} coated gears	Acrylic Acid Gears (no nickel)	Difference
PMP gears	18.2	2920	1504	1416
	1.65	1689	816	873
	0.15	1169	743	426
PMA gears	18.2	3025	3127	-102
	1.65	2231	2107	124
	0.15	1647	1398	249
HDPE gears	18.2	3061	2834	227
	1.65	1653	1542	111
	0.15	1222	1156	66.5

The ELISA results clearly demonstrate the superiority of the PMP base polymer for performance of nickel chelating polyacrylic acid gears.

EXAMPLE 9

SYNTHESIS OF CALCIUM-CHELATING POLYACRYLIC ACID GEARS 5

Gears composed of polyacrylic acid grafted poly-(4-methylpentene-1) are washed with water, 0.1M NaOH, water and then dosed with calcium solution (150mM

21

CaCl$_2$) for 30 minutes. The gears are then washed with water three times. To confirm chelation of calcium to polyacrylic acid graft, calcium-chelated gears are washed with 0.1M aqueous ammonium hydroxide and then reacted with 1% glyoxal-bis(2-hydroxyanil) in 0.1M aqueous ammonium hydroxide solution for 60 minutes. After washing with water, the gears remain red in color.

EXAMPLE 10

USE OF CALCIUM CHELATING POLYACRYLIC ACID GEARS FOR AFFINITY PURIFICATION OF

MITOCHONDRIAL CALCIUM-BINDING PROTEIN

Approximately 100µL of frozen pelleted mitochondria are suspended in 800µL of 10mM Tris-acetate pH 7.0. Two µL of protease inhibitor cocktail (Sigma) are added to the mitochondrial preparation, and the preparation is incubated with calcium-chelating polyacrylic acid gears, prepared as described in Example 9, for 1 hour. The gears are removed and washed with 10mM Tris-acetate pH 7.0 buffer. The bound calcium-binding proteins are eluted from the gears with 8M urea buffer.

EXAMPLE 11

SYNTHESIS OF CONCANAVALIN A-COUPLED LANTERNS

Ten benzaldehyde polystyrene lanterns are incubated for 1 hr at room temperature in a 1M Tris/HCl buffer pH 7.4 containing 0.2%w/v sodium cyano borohydride (Na B H$_3$CN)and Concanavalin A (0.05 mg/mL).

The treated lanterns are then washed 5 times with water, and once with 0.01 M phosphate-buffered saline, pH 7.4 (PBS).

EXAMPLE 12

USE OF CONCANAVALIN A-COUPLED LANTERNS FOR AFFINITY PURIFICATION OF

MITOCHONDRIAL GLYCOPROTEIN

Approximately 100µL of frozen pelleted mitochondria are suspended in 800µL of 10mM Tris-acetate pH 7.0 plus 1 mM CaCl$_2$, 1 mM MgCl$_2$, 1 mM MnSO$_4$. Two

22

μL of protease inhibitor cocktail (Sigma) is added to the mitochondria preparation, and Concanavalin A-coupled lanterns, prepared as described in Example 11, are incubated in the preparation for 1 hour. The lanterns are removed and washed with 10mM Tris-acetate pH 7.0 buffer. The bound glycoproteins are eluted from the lanterns with 8M urea buffer.

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EXAMPLE 13

SYNTHESIS OF POLYSTYRENE SULFONIC ACID LANTERNS 9

Ten polystyrene lanterns were added to a solution of chloroform (20mL). After 10 minutes chlorosulfonic acid (4mL) was added, and the mixture shaken at room

10   temperature for 10 minutes. The lanterns were washed five times with dichloromethane, and dried under vacuum to give polystyrene sulfonic acid lanterns.

EXAMPLE 14

SYNTHESIS OF BROMOPOLYSTYRENE LANTERNS 7

15   Thallium acetate (400mg) was dissolved in 100mL of dry DCM, yielding a yellow solution with some white precipitate. Fifty lanterns (145μMX50; 7.25mmmol) were added into the solution and left shaking for one hour. Bromine (0.5mL, 9.757mmol) was added in 0.1mL portions over a period of 15 minutes. The reaction mixture was left standing at room temperature for one hour, and the solvent decanted off. Lanterns were

20   washed with the following order of solvents: methanol (X3), DCM (X3), warm solution of 20% water in THF (X3), methanol (X2) and DCM (X3). The lanterns were air-dried for one hour under high vacuum (at 25C) for 4 hours to yield bromopolystyrene lanterns.

EXAMPLE 15

25   SYNTHESIS OF ISOCYANATE POLYSTYRENE LANTERNS 4

Triphosgene (198mg) was dissolved in dry DCM (10mL) and gently stirred under nitrogen. Twelve aminomethylated polystyrene lanterns (TFA salt) were added, and left stirring under nitrogen for 10 minutes. The mixture was cooled in an ice bath and after 15 minutes diisopropylethylamine (0.2mL) was added slowly. A further 0.2mL of

23

diisopropylethylamine was added, and the mixture stirred under nitrogen for 30 minutes. The mixture was allowed to warm to room temperature, and stirring was continued for 6 hours. The solution was decanted, and the lanterns washed with DCM (x3), THF (x3), DCM (x3) to yield isocyanate polystyrene lanterns.

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EXAMPLE 16

SYNTHESIS OF HIGH-LOADING AMINOMETHYLATED POLYSTYRENE LANTERNS 1

N-Hydroxymethylphthalimide (120g) was dissolved in 20% trifluoroacetic acid/DCM (2500mL) and methanesulfonic acid (125mL) was added. 5100 Polystyrene

10   lanterns were added, and the mixture was shaken gently for 24 hours. The lanterns were washed with 20% trifluoroacetic acid/DCM, DCM (x2) and methanol. A solution of 5% hydrazine/methanol (3000mL) was added, and the mixture refluxed for 18 hours. The lanterns were washed with hot methanol (x4), 1% trifluoroacetic acid/DCM, and DCM. Drying under vacuum yielded high-loading aminomethylated polystyrene lanterns (TFA

15   salt). The loading was 80 micromole/lantern.

EXAMPLE 17

SYNTHESIS OF PALMITIC ACID-DERIVATIZED LANTERNS

A 0.1M solution of palmitic acid/ diisopropylcarbidimide/1-

20   hydroxybenzotriazole in DMF is added to 10 high-loading aminomethylated polystyrene lanterns, prepared as described in Example 16, and reacted for 3 hours. The lanterns are washed with DMF (x3) and DCM (x3) before been dried under vacuum for 18 hours.

EXAMPLE 18

25   USE OF PALMITIC ACID-DERIVATIZED LANTERNS FOR AFFINITY PURIFICATION OF

MITOCHONDRIAL HYDROPHOBIC PROTEINS

Approximately 100µL of frozen pelleted mitochondria are suspended in 800µL of 5M NH_4OH/10% methanol. Two µL of protease inhibitor cocktail (Sigma) are added to the mitochondria preparation, and palmitic acid-derivatized lanterns, prepared as

24

described in Example 13, are incubated in the preparation for 1 hour. The lanterns are removed and washed with 10% methanol in water. The bound hydrophobic proteins are eluted from the lanterns with 8M urea buffer.

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EXAMPLE 19

SYNTHESIS OF 2-CHLOROTRITYL ALCOHOL LANTERNS 14

Aluminum chloride (0.79g) was mixed with DCM (10mL), and 20 polystyrene lanterns were added. 2-Chlorobenzoyl chloride (1050mg) was added, and the mixture stirred for 7 minutes, followed by 3 hours with no stirring. The lanterns were

10 washed with methanol, 20% water in THF (x2), methanol (x2), and DCM (x3). The lanterns were suspended in 0.8M sodium borohydride in THF, and 2 drops of methanol added. After reacting at room temperature for 18 hours, the lanterns were washed with methanol, 20% water in THF, 20% 2MHCl in THF, 20% water in THF, methanol (x2) and DCM (x3). The lanterns were dried under vacuum for 3 hours to yield 2-chlorotrityl

15 alcohol polystyrene lanterns.

EXAMPLE 20

SYNTHESIS OF REM LINKER LANTERNS 15

Ten Hydroxymethyl D-series Lanterns (initial specified loading, 36μmol)

20 were treated with 5 mL of a solution of DIEA (500μL, 2870μmol, 8 mole equivalents) in DCM. To the mixture was added acryloyl chloride (260μL, 2870μmol, 8 mole equivalents). The reaction was allowed to stand at rt for 4 hours. The reagent solution was decanted and the Lanterns washed with DMF (3x3min) and DCM (3x3min).

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EXAMPLE 21

SYNTHESIS OF MORPHOLINO LANTERNS 16

A 0.1M solution of morpholine in dichloromethane (5mL) was added to 10 SPPSDREM lanterns and reacted overnight at room temperature. The reagent solution was decanted and the Lanterns washed with DMF (3x3min) and DCM (3x3min).

EXAMPLE 22

SYNTHESIS OF TRIPHENYLPHOSPHINE LANTERNS 11

Under an atmosphere of nitrogen, a 0.1M solution of chlorodiphenylphosphine in dry THF 5mL) was added to 10 lithiated polystyrene lanterns

5   6. After reaction for 3hrs, the lanterns were washed with dichloromethane (2 x 10 mins), followed by hot 20% H_2O/THF (1 hour), and soaked in 20%H_2O/THF overnight, then soaked in dichloromethane (30 min), and air dried.

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration,

10   various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.